UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

10

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/249,543	02/12/99	EVANS T	NEB-154

028986
NEW ENGLAND BIOLABS, INC.
32 TOZER ROAD
BEVERLY MA 01915

HM12/0713

EXAMINER	
MOORE, W	
ART UNIT	PAPER NUMBER
1652	7

DATE MAILED: 07/13/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trad marks

Office Action Summary	Application No. 09/249,543	Applicant(s) EVANS ET AL.	
	Examin r William W. Moore	Art Unit 1652	

-- Th MAILING DATE of this communication appears on the cover sheet with th correspond nc address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *22, 23, 29 and 30* is/are allowed.

6)☒ Claim(s) *1-21, 24, 25, and 27-28* is/are rejected.

7)☒ Claim(s) *26* is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

Attachment(s)

15) ☒ Notice of References Cited (PTO-892)
16) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 2 .
18) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other:

DETAILED ACTION

Specification

Applicants' submission of an Information Disclosure Statement, Paper No. 2 filed April 5, 1999, is hereby acknowledged. A Notice of Draftsman's Patent Drawing Review, stating informalities requiring correction, accompanies this communication. Claims 1-30 are examined herein.

Claim Rejections - 35 USC § 112

Claims 1-4, 6, 8-12, 15-19, 21, 24, 25, 27 and 28 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for methods for fusing target proteins initially generated by cleavage of intein-comprising precursor proteins **wherein a second target protein, or region, in the method has an amino-terminal cysteine**, and for a resulting, ligated, fusion protein, whether linear, cyclic, or polymeric, does not reasonably provide enablement for methods for fusing target proteins, or for the resulting fusion polypeptides, wherein a second target protein used in a fusion method has no amino-terminal cysteine. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The specification does not disclose, nor does the prior art of record herein otherwise provide, a method a method for ligating two target proteins, or for fusing separate regions of a single polypeptide to form a cyclic polypeptide, wherein ligation is accomplished with a second target protein, or a second region of a single polypeptide, lacks an amino-terminal cysteine. The specification and the prior art, e.g., Muir et al., submitted with Applicant's Information Disclosure Statement and Dawson et al. and Severinov et al., made of record herewith disclose that "ligation" is a chemical process distinct from native excision of an intein and concomitant fusion of flanking exteins. The specification and the prior art teach that a chemical "ligation" process provides the advantage of separately producing a future fusion partner absent an intein for ligation with another fusion partner upon contact in solution. More specifically, if a first fusion partner has the C-terminal thioester required by claims 1, 8, 16, 17 and 22 herein, Dawson et al. teach, p. 777, that "chemical ligation is limited to reaction at an amino-terminal Cys residue" of the second fusion partner and

Severinov et al. teach, p. 16207, "that no ligation was detected using a control [second fusion partner] that did not contain an N-terminal cysteine residue". While Muir et al. teach that a first fusion partner may have different forms of C-terminal thioesters, they teach only the ligation of a second fusion partner having an N-terminal cysteine because only a nucleophilic attack by the thiolate of the second fusion partner's terminal cysteine can release a generic thioester to permit formation of a peptide bond between the terminal carboxyl of the first fusion partner and the amino group of the second fusion partner's terminal cysteine. Nothing in the record suggests that ligation would occur if cysteine were replaced by, e.g., serine or threonine, weaker nucleophiles not shown to capable of releasing a generic thioester at the carboxyl-terminus of a first target polypeptide and cysteine is the only amino acid the specification or prior art disclose or suggest as the "specified" amino-terminal of a second fusion partner.

It is well settled that 35 U.S.C. §112, first paragraph, requires that a disclosure be sufficiently enabling to allow one of skill in the art to practice the invention as claimed without undue experimentation and that unpredictability in an attempt to practice a claimed invention is a significant factor supporting a rejection under 35 U.S.C. §112, first paragraph, for non-enablement. See, *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (applying the "Forman" factors). Cf., *Ex parte Forman*, 230 USPQ 546, 547 (Bd. Pat. App. & Int. 1986) (citing eight factors relevant to analysis of enablement). The standard set by the CCPA is not to "make and screen" any and all possible alterations because a reasonable correlation must exist between the scope of guidance the specification provides and the scope asserted in the claimed subject matter. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 25 (CCPA 1970) (scope of enablement varies inversely with the degree of unpredictability of factors involved in physiological activity of small peptide hormone). The Federal Circuit approved this standard of the CCPA in *Genentech, Inc. v.*

Novo-Nordisk A/S, 42 USPQ2d 1001 (Fed. Cir. 1997). Applying the "*Forman*" factors discussed in *Wands*, to Applicant's disclosure, it is apparent that:

a) the specification lacks adequate, specific, guidance for practice of a claimed method with a required first fusion partner having a C-terminal thioester where the second fusion partner lacks an amino-terminal cysteine,

b) the specification lacks working examples wherein a claimed method can be practiced with a second fusion partner lacking an amino-terminal cysteine,

c) in view of the prior art publications of record herein, the state of the art and level of skill in the art do not support replacement of an amino-terminal cysteine of the a second fusion partner with any other naturally-occurring amino acid in a method requiring a first fusion partner have a C-terminal thioester, and,

d) unpredictability exists in the art where no amino acid but cysteine can provide a sulfhydryl-bearing functional group capable of releasing a thioester present at the carboxyl terminus of a first fusion partner as required by the claimed methods.

Thus the scope of the subject matter embraced by the phrase, "specified N-terminal", is unsupported by the present specification even if combined with the teachings available in the prior art. This rejection may be overcome by limiting methods of claims 1, 8, 16 and 17, to "generating a [second] ligation target protein [or region] having an N-terminal cysteine", and by limiting a modified intein product of claim 24 to an intein capable of "cleavage to produce a cysteine at the N-terminal of an adjacent target protein". Claims 7, 14, 20 and 26 would therefore become redundant and may be canceled.

Claims 2-14, 16-20, 28 and 29 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Plasmids disclosed in the specification and commonly used in the art are all composed of polymers of nucleic acids. None comprise amino acids, peptides or proteins. Inteins, exteins, peptides and proteins disclosed in the specification are all composed of amino acid polymers having no covalent, or other, association with any nucleic acids, an art-recognized feature of proteins. Yet each of claims 2, 8, 16, 17, 28 and 29 suggests that a method might be practiced with one or more plasmids "comprising" an intein or comprising a protein having an intein. This unusual terminology does not accord with any description of

plasmids in the relevant arts of molecular biology and protein engineering, rendering each of claims 2, 8, 16, 17 and 28 ambiguous and indefinite. Claims 3-7, 9-15, and 18-20 are included in this rejection because they depend from claims 2, 8, 16 and 17 but describe methods without correcting or otherwise clarifying the ambiguity of claims from which they depend.

Applicant actually intends to describe methods utilizing **plasmids comprising nucleic acid sequences**, or **polynucleotides**, that **encode a protein comprising a first intein, or a second intein**. Indeed, no target protein can be generated until a first intein region, or a second intein region, is cleaved from an adjacent extein region, a process that claims 4, 12, and 19 commemorate in their numbered subscripts "-1" and "1" describing amino acids flanking the site of intein/extein cleavage. Amending claims 1 or 2, claims 8 or 9, and each of claims 16, 17, 28 and 29 so that they describe the art-recognized relationship - stated in claim 30 - between an encoding nucleic acid sequence, e.g., in a plasmid, and an encoded amino acid sequence will overcome this rejection.

Claim Rejections - 35 USC §102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. §102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

Claims 1 and 15 are rejected under 35 U.S.C. §102(a) as being anticipated by Muir et al., 1998, **Proceedings of the National Academy of Sciences, USA**, Vol. 95, pages 6705-6710.

Muir et al. disclose, three months before the filing date of Applicant's provisional application serial No. 60/102,413, a method for fusing a recombinantly expressed target protein having a carboxyl-terminal thioester provided by thiophenol-induced cleavage of an intein, to a second target protein having an amino-terminal cysteine, see Figure 2 and text at pages 6706-6707, wherein the first and the second target proteins are then chemically

ligated by a nucleophilic attack of the amino-terminal cysteine of the second target protein upon the carboxyl-terminal thioester of the first target protein, meeting limitations of claim 1 which does not require recombinant expression of either or both target proteins. Muir et al. also disclose the resulting ligated fusion protein, meeting limitations of claim 15.

Claims 1 and 15 are rejected under 35 U.S.C. §102(a) as being anticipated by Severinov et al., 1998, **The Journal of Biological Chemistry**, Vol. 273, pages 16205-16209.

Severinov et al. disclose, three months before the filing date of Applicant's provisional application serial No. 60/102,413, a method for fusing a recombinantly expressed first fusion protein target to a second target protein having an amino-terminal cysteine wherein the first target protein has a carboxyl-terminal thioester provided by thiophenol-induced cleavage of an intein which is associated either a 66-amino acid target or a 568-amino acid target, see Figures 2 and 3 text at pages 16207, right column, through 16209, left column. Severinov et al. further disclose that the first and the second target proteins are chemically ligated by a nucleophilic attack of the amino-terminal cysteine of the second target protein upon the carboxyl-terminal thioester of the first target protein, meeting limitations of claim 1 which does not require recombinant expression of either or both target proteins. Severinov et al. also disclose the resulting, ligated, 70kd fusion protein of 602 amino acids, meeting limitations of claim 15.

Claims 24 and 28 are rejected under 35 U.S.C. §102(a) as being anticipated by Chong et al., 1998, **The Journal of Biological Chemistry**, Vol. 273, pages 10567-10577.

Chong et al. disclose, see pages 10572-10573 and accompanying Table IV and Figs. 4 and 5, the preparation of a modified intein comprising a mutant intein, having the amino acid substitution H453Q and capable of pH and temperature-induced *in vitro* cleavage between the intein C-terminus and the adjacent extein N-terminus producing a specified residue, alanine, at that N-terminus. Chong et al. also disclose, page 10568, preparation of a plasmid, pMYT4, wherein a DNA sequence encodes the modified, mutant, intein.

Claim Rejections - 35 USC §103

The following is a quotation of 35 U.S.C. §103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. §103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR §1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. §103(c) and potential 35 U.S.C. §§102(f) or (g) prior art under 35 U.S.C. §103(a).

Claims 2, 7, 8-10 and 14 are rejected under 35 U.S.C. §103(a) as being unpatentable over either of Muir et al. or Severinov et al., discussed above, in view of Comb et al., U.S. Patent No. 5,496,714, and Mills et al., 1998, **Proceedings of the National Academy of Sciences, USA**, Vol. 95, pages 3543-3548.

The teachings of Muir et al. and Severinov et al. are taken as before and the further teachings of both Muir et al. and Severinov et al. of the use of plasmid expression vectors and transformed host cells for the recombinant expression of a fusion polypeptide wherein the plasmid expression vectors comprise a nucleic acid sequence encoding a first target polypeptide fused at its carboxyl terminus to the amino terminus of an intein element, itself fused to the amino terminus of an affinity tag polypeptide, specifically, a chitin-binding domain. Both Muir et al. and Severinov et al. also teach that, after recombinant expression in a host cell transformed with the plasmid, they purified the first target protein fused to an intein element and a chitin binding domain by lysing the host cell and passing the lysate through a chitin resin column. Both Muir et al. and Severinov et al. then teach that thiol reagent-induced cleavage releases the first target protein from the intein element and chitin-binding domain portion of the expressed fusion polypeptide, and provides the first target protein with a C-terminal thioester "tag". Muir et al. and Severinov et al. both

further teach that a second polypeptide having an N-terminal cysteine can readily be ligated to the first target protein, attacking its C-terminal thioester to form the peptide bond linking the first and second target polypeptides to form a new fusion protein.

Thus either of Muir et al. and Severinov et al. teach all elements of claims 1, 2, 8-10 and 14 with regard to a first plasmid, a first target protein, its purification and its C-terminal thioester tag but do not teach the recombinant expression of a second target protein comprised within an intein-associated fusion protein encoded by a plasmid-borne nucleotide sequence. Consequently, the teachings of both Comb et al.('714), available as prior art under 35 U.S.C. §102(b), and of Mills et al., available as prior art under 35 U.S.C. §102(a), are combined with the teaching of either of Muir et al. or Severinov et al. Comb et al. teach, cols. 8 and 9 and claims 34 and 35, that a first target protein comprising a first intein may be placed in solution with a second target protein comprising a second intein under conditions wherein the first and second inteins are excised and the first and second target proteins concurrently spliced, and also teach that both intein-target protein fusions may be encoded by nucleic acid sequences borne by separate plasmids and recombinantly-expressed in separate host cells. Mills et al. teach the use of a mutant intein in an *in vitro* method for trans-splicing, see pages 3543 and 3544 and Fig. 8, induced by adding dithiothreitol [DTT] and raising the temperature of the *in vitro* solution to 25°C, whereby splicing provides a new fusion protein from a first recombinantly expressed target protein and a second recombinantly expressed target protein, and wherein the first target protein comprised an amino-proximal target protein fused to a carboxyl-proximal mutant intein and the second target protein comprised an amino-proximal mutant intein fused to a carboxyl-proximal target protein. Mills et al. teach, page 3548, that their "results show that the N- and C-terminal intein fragments essentially constitute a polypeptide ligase system that allows the *in vitro* ligation of any two proteins fused to such fragments".

It would have been obvious to one of ordinary skill in the art to substitute a recombinantly-expressed second target protein which is a fusion of an amino-proximal mutant intein and a carboxyl-proximal target protein according to Mills et al. and has an amino-terminal cysteine according to either of Muir et al. or Severinov et al. instead of the chemically-synthesized second target protein used by either Muir et al. or Severinov et al. in an *in vitro* method for fusion of first and second target proteins utilizing the first target protein of either of Muir et al. or Severinov et al. liberated by thiol reagent-induced cleavage from a fusion of an expressed amino-proximal target protein fused to a carboxyl-proximal intein and having a C-terminal thioester available for formation of a peptide bond with N-terminal cysteine of the second target protein. This is because Comb et al.('714) teach that a first and a second intein in separate fusion proteins should be excised *in vitro* while a first and a second target protein joined, respectively, to the first and second intein are concurrently spliced *in vitro* to form a new fusion protein, because either of Muir et al. or Severinov et al. teach how such splicing may be controlled and expedited by using thiol reagent-induced cleavage to liberate the first target polypeptide from the first intein to permit formation of a peptide bond with a nucleophilic attack upon the resulting thioester by an amino-terminal cysteine of the second target protein, and because Mills et al. teach that mutant inteins may be used to promote the *in vitro* splicing of two recombinantly expressed target proteins encoded by separate nucleic acid sequences as fusion proteins each comprising a mutant intein borne by plasmid vectors.

Allowable Subject Matter

Claims 22, 23, 29 and 30 are allowed. Claim 26 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. The subject matters of methods of claims 3-6, 11-13 and 16-20, of the polymeric and cyclic protein

and modified intein products of claims 21-23, 25-27, and of the plasmid-borne nucleic acid coding regions of claims 29 and 30, are free of the prior art of record which fails to suggest that one of ordinary skill in the art at the time the invention was made should use trans-splicing systems to produce cyclic or polymeric polypeptides. Neither is there any suggestion in the prior art to select, to modify, and to insert the *M. thermoautotrophicum* ribonucleoside-diphosphate reductase intein, see Figure 8 of Smith et al., made of record with Applicant's information disclosure statement, into any protein fusion partner in order to practice a method of claims 3-6, 11-13 and 16-20, to produce a protein of claim 21, to provide an intein of claims 22, 23 and 25-27, or to be encoded by a DNA segment of claim 30 that may be comprised by a plasmid of claim 29, particularly where other modified inteins had already been used by Chong et al.(1998) and Xu et al.(1996), both made of record with Applicant's information disclosure statement, and by Telenti et al., made of record herewith. Claims 3-6, 11-13, 16-21, 25 and 27 would therefore also be allowable if rewritten to overcome the rejections under 35 U.S.C. §112 set forth in this Office action and to include all limitations of the base claim and any intervening claims.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is 703.308.0583. The examiner can normally be reached between 8:00AM-6:30PM EST on Tues./Thurs./Fri. and between 11:30AM-6:00PM on Mon./Wed. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy can be reached on 703.308.3804. The fax phone numbers for the organization where this application or proceeding is assigned are 703.308.4242 for regular communications and 703.308.0294 for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703.308.0196.

William W. Moore
July 11, 2001

PONNATHAPU ACHUTAMURTHY
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600